small differences observed were sufficient to influence Th cell differentiation via altered mTORC1-mTORC2 and Akt-PKC-θ activation. Moreover, the strength of the CD3 and CD28 signal might be important for selective mTOR pathway activation or other environmental factors influence the Th cell differentiation process via mTOR signaling. Finally, as nutrients such as amino acids or glucose directly control the activation of the mTOR pathway, it is tempting to hypothesize that nutrients do not merely control T cell proliferation but that the nutritional status might also control Th cell differentiation via the mTOR signaling complex.

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Only the Strong Survive

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Whether apoptosis is relevant for interclonal competition of T cells after antigen encounter has remained uncertain. In this issue of *Immunity*, Wensveen et al. (2010) establish a critical role for the proapoptotic BH3-only protein Noxa in this selection process.

Apoptosis plays a critical role in the selection of functionally competent immune cells. During lymphocyte development, apoptosis eliminates cells that lack antigen receptor expression in a process known as "death by neglect," but also mediates the death of cells that express potentially autoreactive antigen receptors during "negative selection," critical for generating tolerance. Both types of cell death are controlled by *B* cell *l*ymphoma-2 (Bcl-2) family proteins that fall into pro- and antiapoptotic groups and that integrate different forms of cell stress as well as developmental cues at the level of mitochondria in order to preserve cell survival or initiate cell death (Strasser, 2005). Apoptosis has also been recognized as critical for affinity maturation of B cells competing for a limited amount of antigen and supporting cytokines in germinal center reactions. This process secures that only clones expressing B cell receptors with the highest affinity are able to thrive. Bcl2l11, also known as *B*cl-2 *i*nteracting *m*ediator of cell death (Bim), a BH3-only protein belonging to one proapoptotic subclass of the Bcl-2 family with at least eight members, has been shown to be critical for this selection process (Fischer et al., 2007). Bim is also critical for T cell deletion after antigen clearance in which other BH3-only proteins, such as Puma or Noxa (also known as Pmaip1), can play auxiliary roles (Bauer et al., 2006). Notably, the latter two proteins have been identified as direct target genes of the tumor suppressor *p53*, activated in order to execute apoptosis induced by DNA damage. However, the physiological role of Noxa has remained rather obscure, given that its loss confers only mild resistance to DNA-damage-induced apoptosis, and only in selected cell types (Ploner et al., 2008).

One of the first hints that Noxa can act outside the DNA-damage response came from the demonstration that its expression was induced in response to TCR ligation in human naive T cells and that repression of protein expression by RNA interference provided a selective advantage in cell-growth competition experiments to Jurkat T acute lymphoblastic leukemia and primary activated T cells under conditions of glucose limitation (Alves et al., 2006). Others observed that Noxa limits the lifespan of memory T cells (Yamashita et al., 2008) or, together with Bim, restricts survival of NK cells in the absence of their major survival cytokine, IL-15 (Huntington et al., 2007). Performing a consequent follow up of their initial observations, Wensveen et al. (2010) now provide compelling evidence that Noxa limits the survival of T cell clones that express low-affinity TCRs during interclonal competition, a process that leads to the outgrowth of the "best-fit" high-avidity T cell clones from a large panel of antigen-reactive naive T cells during antigenic challenge (Malherbe et al., 2004; Zehn et al., 2009).

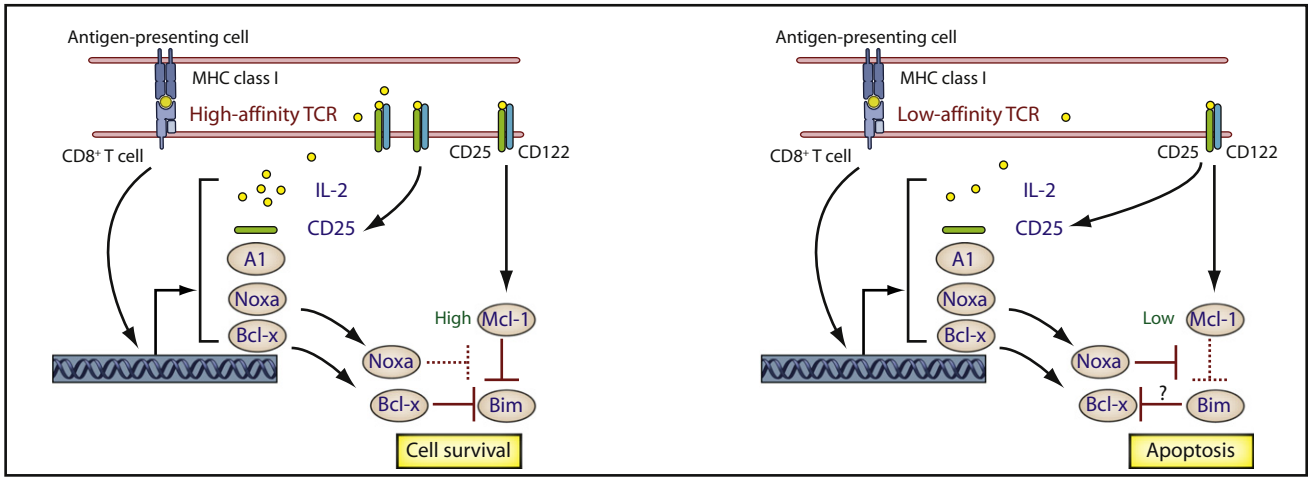

Figure 1. Noxa Sets the Threshold for T Cell Apoptosis during Interclonal Competition
High-affinity TCR ligation induces transcription of genes relevant for cell growth and survival (e.g., *Il2*, *Bcl2l1*, and *Bcl2a1a*). Secreted IL-2 drives autocrine induction of its receptor components. Noxa expression rises gradually over time leading to Mcl-1 codegradation that is compensated by IL-2 receptor-mediated increases of Mcl-1 protein, sufficiently high to also neutralize proapoptotic Bim. In response to low-affinity TCR ligation, IL-2 and subsequent CD25 production are insufficient to maintain high-enough Mcl-1 levels that drop below threshold leading to a relative increase in free Bim that triggers apoptosis.

Expression analysis of 40 preselected genes involved in cell death signaling revealed that Noxa mRNA gradually increases upon TCR ligation in mouse $CD8^+$ T cells. This increase was separated well in time from the rapid increase of mRNAs encoding the prosurvival Bcl-2 family proteins Bcl-xL and Bfl1/A1 and was paralleled by a posttranslational increase in proapoptotic Bim and antiapoptotic Mcl-1. Notably, after Noxa accumulation reached a plateau, amounts of Mcl-1, its preferred binding partner within the Bcl-2 family and most critical for peripheral T and B cell survival, declined (Strasser, 2005). This observation is consistent with the fact that Noxa-Mcl-1 interaction initiates their proteasomal codegradation (Ploner et al., 2008). Keeping in mind that Mcl-1 reportedly neutralizes Bim by direct binding in unstressed cells, they investigated possible quantitative differences in Mcl-1-Bim complexes in wild-type and *Pmaip1*$^{-/-}$ T cells after TCR ligation. Consistent with one model of BH3-only protein action, Bim was found in complex with Mcl-1 for an extended period of time in the absence of Noxa, suggesting that Bim may become activated less efficiently.

A long-term follow up of *Pmaip1*$^{-/-}$ mice revealed accumulation of $CD8^+$ $CD44^+CD62L^{low}$ effector T cells in aged animals. However, because loss of Noxa does not to interfere with T cell development and homeostasis or causes autoimmune-related pathology (Ploner et al., 2008), the authors considered the possibility that it may be critical earlier on during expansion and interclonal selection of antigen-activated T cells. This idea was tested by infection of wild-type and *Pmaip*$^{-/-}$ mice with influenza virus A/PR8/34, and expansion of $CD8^+$ effector T cells was monitored over time. Surprisingly, although the percentage of effector T cells was mildly elevated in *Pmaip*$^{-/-}$ animals, the percentage of high-affinity T cells, specific for the major influenza epitope $D^bNP_{366\text{-}374}$, was actually lower. As a consequence, IFN-γ production was reduced and knockout animals recovered from infection significantly slower than wild-type controls.

The CTL response against influenza is usually dominated by one or two high-affinity $V\beta8.3^+$ clones with well-defined complementarity determining regions (CDRs) 3, indicative for strong selection processes during T cell expansion. When antigen-specific T cells were sorted at the peak of expansion on the basis of tetramer staining and subjected to TCR spectratyping and sequencing, Noxa-deficient antigen-specific T cells showed similar clonal expansion but much higher TCR diversity. Such results suggested that in the absence of Noxa, T cell clones with suboptimal TCRs survive and compete for survival factors with other, more specific T cell clones. This situation is reminiscent to the one found in germinal centers in *Bcl2l11*$^{-/-}$ mice where B cells evade the selection pressure during affinity maturation, show limited somatic hypermutation, and produce mainly low-affinity antibodies (Fischer et al., 2007).

For showing that the observed effects were T cell autonomous, wild-type and *Pmaip*$^{-/-}$ T cells were transferred into a mouse model that expresses the costimulatory ligand CD70 as a transgene on B cells, driving antigen-dependent polyclonal T cell expansion, but simultaneously also lacks its receptor, CD27. In this environment, Noxa-deficient T cells expanded much more rapidly than their wild-type counterparts and showed a $KLRG1^+$ effector cell phenotype, indicative for their descent from recently activated T cells. In contrast, their contraction due to exhaustion of naive precursor cells appeared unaffected by loss of *Pmaip1*. For showing that the effect on T cell expansion required TCR-signaling and related affinity thresholds, $CD8^+$ T cells from OT-I and OT-I mice lacking *Pmaip1* were transferred into isogenic recipients, subsequently infected with low- or high-affinity peptide-expressing *Listeria monocytogenes* strains. Antigen-specific T cell expansion did not differ between genotypes when it was challenged with a high-affinity peptide, whereas Noxa-deficient OT-I cells performed much better than their wild-type OT-I counterparts when challenged with low-affinity peptide, a phenomenon also recapitulated in cell culture.

So, how does loss of Noxa facilitate expansion of low-affinity T cell clones (Figure 1)? Interclonal competition has been previously ascribed solely to qualitative differences in the proliferative response of low- versus high-affinity TCR expressing T cell clones (Zehn et al., 2009). Consistently, initial proliferation rates triggered by high- versus low-affinity peptides in OT-I T cells appeared comparable and CD25 levels were found less induced upon low-affinity peptide stimulation. Most critically, this effect associated with reduced Mcl-1 levels that were restored by adding exogenous IL-2, in line with a role for common γ_c-chain-mediated cytokine signaling in maintaining Mcl-1 expression (Huntington et al., 2007). Furthermore, in the absence of Noxa, the decline of Mcl-1 appeared delayed, pointing toward a critical role of the Noxa-Bim-Mcl-1 axis in apoptosis induction during interclonal T cell competition.

Intriguingly, quantitative differences in cell death rates between the genotypes were not reported by the authors, suggesting that these may be minor, as also noted for DNA-damage induced apoptosis in the absence of Noxa (Ploner et al., 2008). Therefore, biological consequences may become evident only gradually, e.g., during extended in vitro culture, upon adoptive transfer into *Cd70*^{tg}*Cd27*$^{-/-}$ mice or in aged *Pmaip*$^{-/-}$ animals. Similarly, possible qualitative differences in cell-cycle activity of Noxa-defective T cells at later stages of antigenic challenge, in which low-affinity TCR expressing clones reportedly stop to thrive (Zehn et al., 2009), were not directly excluded. Further questions arise, e.g., how critical is the observed displacement of Bim from Mcl-1 for death—does it activate Bax, a member of the second class of proapoptotic Bcl-2 family proteins, directly to kill, or is it needed to neutralize Bcl-xL? Is Bim indeed a critical effector during interclonal selection, as established, e.g., in response to cytokine-deprivation or during negative selection (Strasser, 2005). Analysis of interclonal competition in *Bcl2l11*$^{-/-}$ and *Bcl2l11*$^{-/-}$*Pmaip1*$^{-/-}$ mice seems warranted. What are the signaling intermediates downstream of the TCR inducing Noxa expression? It also remains unresolved whether Noxa acts exclusively on Mcl-1 to promote cell death or whether Bfl1/A1, also upregulated under these conditions, needs to be neutralized, too. Finally, whether Noxa is also critical for interclonal selection of different T helper cell subtypes needs to be tested.

Besides all these unresolved issues, opening exciting and novel avenues of research, these observations do have implications for immuno-suppressed patients and the elderly. Their inability to mount effective immune responses may not only be due to ineffective T cell activation, but possibly also due to failed or compromised competition caused by defects in the rheostat of involved Bcl-2 family proteins. Hence, nonselective polyclonal T cell activation may be a suboptimal treatment strategy. Also, BH3 mimetics, nonpeptidic compounds developed to kill tumor cells, designed to selectively mimic the action of Noxa may be a useful tool to aid antiviral therapies or could act as a new type of adjuvant, improving common vaccination strategies.

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